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SEROLOGIC SURVEY FOR *TOXOPLASMA GONDII* AND *NEOSPORA CANINUM* IN THE COMMON BRUSHTAIL POSSUM (*TRICHOSURUS VULPECULA*) FROM URBAN SYDNEY, AUSTRALIA

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ABSTRACT: The common brushtail possum (*Trichosurus vulpecula*) has well adapted to increasing urbanization, resulting in greater interaction with humans and their domestic pets. Wildlife species in urban areas face a higher risk of exposure to zoonotic pathogens and may be affected by parasites hosted by cats (*Toxoplasma gondii*) or dogs (*Neospora caninum*), yet it is unknown to what extent urban *T. vulpecula* are exposed to these parasites. Antibodies to *T. gondii* and *N. caninum* were assayed in sera of 142 adult possums from the city of Sydney, Australia. Using the modified agglutination test, antibodies to *T. gondii* were found in 9 (6.3%) of the 142 animals in titers of 1:25 (4), 1:50 (1), 1:100 (1), 1:800 (1), 1:3,200 (1), 1:6,400 (1), and 1:12,800 (1). Of some *T. vulpecula* multiple sera samples within a 2-yr frame could be collected, but seropositive animals in general were not recaptured after initial seroconversion. One possum had a high *T. gondii* titer on 2 consecutive bleedings, 14 mo apart, and seropositive possums appeared normal when captured. Sex seemed not to have an affect on antibody prevalence, but age and location may play a role. Antibodies to *N. caninum* were not detected in 1:25 dilution of sera in the *N. caninum* agglutination test, indicating that *T. vulpecula* may not have been exposed to this parasite. This is the first serological survey for *T. gondii* and *N. caninum* infections in urban *T. vulpecula*.

The common brushtail possum (*Trichosurus vulpecula*) is a solitary, nocturnal, general herbivorous arboreal marsupial in its native Australian environment (Kerle, 2001). This cat-sized phalanger generally occurs where there are trees, but has disappeared from large parts of its former natural habitat, including all of arid Australia (Goldingay and Jackson, 2004). Declines are attributed to a combination of factors, including habitat loss and disturbance, disease, drought, and impact of predators on depleted populations. Conversely, possums are quite common in many Australian cities, and *T. vulpecula* is a major introduced pest in New Zealand, damaging native forests and spreading bovine tuberculosis (Montague, 2000). This creates distinctive management issues for *T. vulpecula*. Our research focuses on Australian urban areas where they cohabitate with people and their domestic pets, taking up residence in house roofs and browsing on garden plants (Eymann et al., 2006). Wildlife species that live in urban areas are increasingly likely to come into contact with both domestic pets and humans, suggesting the possibility of disease transmission. Emerging infectious diseases are associated with a range of factors including the interaction of zoonotic pathogens with wildlife, domestic animals, and human populations (Daszak et al., 2000; Brown, 2004). Emerging infectious diseases of wildlife include ''spillover'' from domestic animals to wildlife populations living in close proximity and also may be directly related to human intervention, via host or parasite translocations. Many infectious organisms originate from humans and their companion animals (Fayer et al., 2004), and Australians keep at least 1.25 million cats and 1.5 million dogs (English, 1982).

Toxoplasma gondii is among the most frequently reported parasites of humans and animals worldwide (Dubey and Beattie, 1988; Tenter et al., 2000; Dubey and Odening, 2001). This coccidium uses felids as the definitive host and warm-blooded animals as intermediate hosts. Oocysts are shed only in the feces of infected cats. Australian marsupials have evolved in the absence of *T. gondii* and have only recently been exposed to the parasite as there were no cats in Australia before European settlement (Dubey and Beattie, 1988). This makes marsupials highly susceptible for toxoplasmosis, and infection can prove fatal in captive and free-ranging populations. It has serious implications for zoological gardens exhibiting susceptible animals, such as kangaroos, in close proximity to felids (Spencer et al., 2003), and there are numerous reports of deaths in zoos (reviewed in Dubey and Odening, 2001). Toxoplasmosis may remain clinically inapparent, causing sudden death without clinical signs in animals that often have good body condition (Canfield et al., 1990; Obendorf and Munday, 1990). If stress reduces immunocompetence, the parasite may multiply and cause a range of symptoms, including lethargy, unnatural daytime activity, inappetence, respiratory distress, and neurological disturbances. Nutritional and weather stresses are considered possible factors causing latent *T. gondii* infection to become clinically obvious and subsequently fatal (Obendorf and Munday, 1983). *Toxoplasma gondii* infection has been found in a number of wild Australian marsupials, including macropods (Johnson et al., 1988, 1989), eastern barred bandicoots (*Perameles gunnii*) (Obendorf et al., 1996), quokkas (*Setonix brachurus*) (Gibb et al., 1966), dasyurids (*Dasyuroides byrnei*) (Attwood et al., 1975), and wombats (*Vombatus ursinus*) (Hartley and English, 2005). *Trichosurus vulpecula* previously has been diagnosed with toxoplasmosis (Cook and Pope, 1959; Presidente, 1984; Canfield et al., 1990; Viggers and Spratt, 1995), including possums from the Sydney metropolitan area (Obendorf et al., 1998); Hartley (1993) found *T. gondii* encephalitis in an unknown number of possums held at Taronga Zoo Pathology, Sydney. However, the prevalence of *T. gondii* in possum populations remains unknown. A serological survey of *T. vulpecula* from Kangaroo Island, South Australia, detected no antibodies to *T. gondii* in 30 possum sera tested (Callaghan and Moore, 1986). Cats may contaminate urban areas, which places *T. vulpecula* at risk, as they commonly feed on the ground (MacLennan, 1984). Possums may be infected by accidental ingestion of food or water contaminated with oocysts from infected cat feces.

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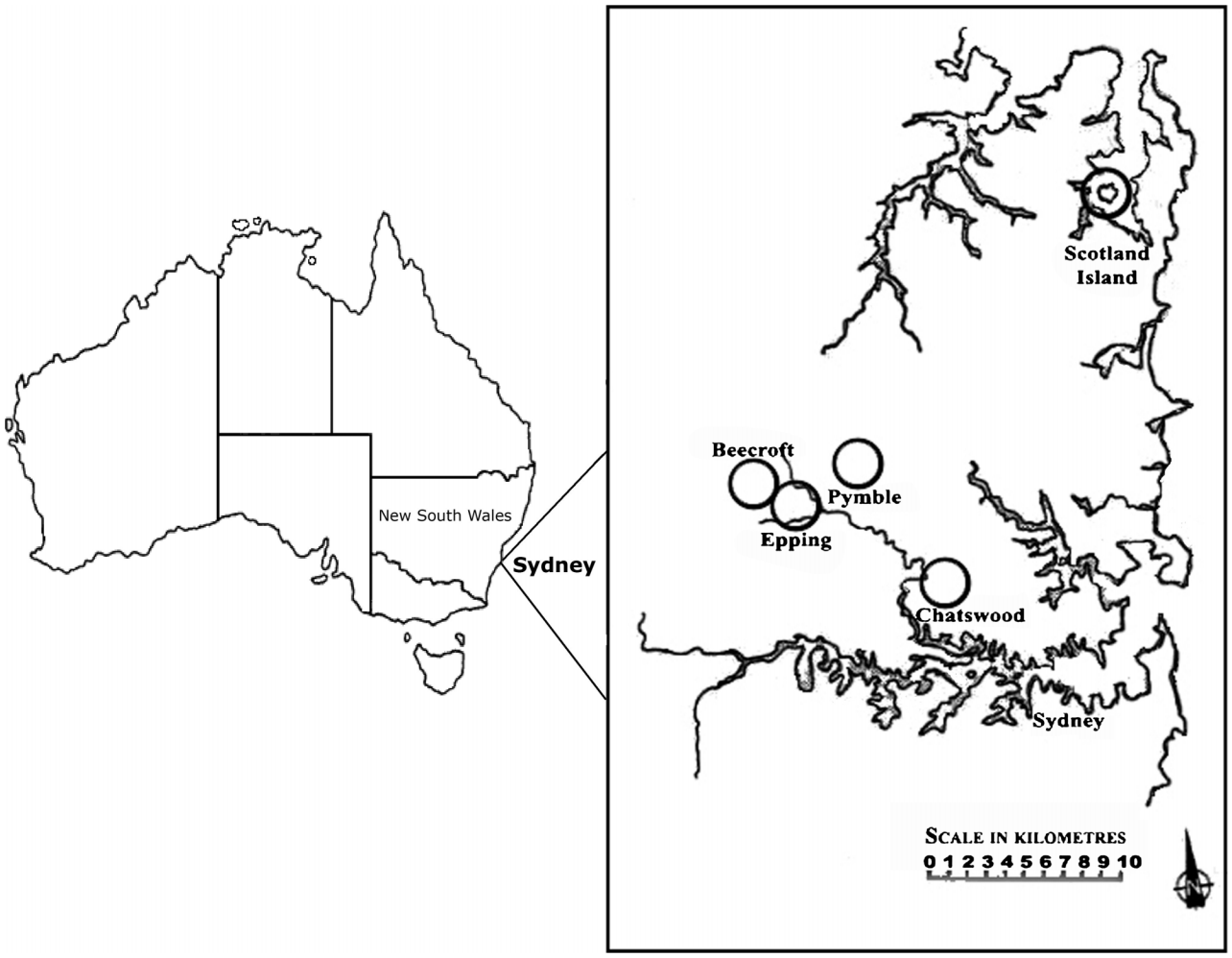

FIGURE 1. Map of Australia showing Scotland Island and 4 Sydney metropolitan suburbs in New South Wales—Beecroft, Chatswood West, North Epping and Pymble—from which *Trichosurus vulpecula* was sampled.

Neospora caninum is a recently recognized coccidian biologically similar to *T. gondii* (Dubey et al., 1988; Dubey, 2003). The dog (*Canis domesticus*) and the coyote (*Canis latrans*) are its definitive hosts (McAllister et al., 1998; Gondim et al., 2004). Although there are reports of *N. caninum* infections in many species of animals (for review see Dubey, 2003), including the South American opossum (*Didelphis marsupialis*) (Yai et al., 2003), it has not been reported in possums yet. The objective of this study was to investigate seroprevalence of *T. gondii* and *N. caninum* in urban *T. vulpecula* and to analyze any association between seroprevalence and sex, age, body condition, and geographic location.

MATERIALS AND METHODS

Collection of samples

Trichosurus vulpecula blood samples were collected in the garden area of 35 residential houses from 5 localities within the Sydney metropolitan area, New South Wales, Australia (Fig. 1). Locations included 4 North Shore mainland suburbs (Beecroft, Chatswood West, North Epping, and Pymble) and Scotland Island, a 52 ha land mass located at Pittwater. The environment of Scotland Island is similar to that of the sampled mainland suburbs, with high residential levels and dense surrounding vegetation including many trees. Attempts were made to recapture possums in consecutive trap nights on 4 different occasions between November 2002 and April 2005 to allow repetitive monitoring. Seven samples from an opportunistic field trip to Myall Lake National Park, NSW also were included in this study. In total, 195 blood samples from 142 individuals of both sexes and varying ages were collected.

Possum handling

Animals were caught overnight in possum cage traps (0.6 m × 0.3 m × 0.3 m) baited with apple and peanut butter and were examined the following morning (Duckworth and Meikle, 1995). Possums were physically restrained in hessian bags while anesthetic was administered into the gluteal muscle using a combination of ketamine (20 mg/kg) and xylazine (2 mg/kg). They were microchipped, sexed, aged, and weighed, and general appearance was noted. Individuals were classified as adult if they were 2 kg or higher and had class 2 or higher teeth wear (Winter, 1980; Cowan and White, 1989). Tooth wear gives only an indication of the possum's age; for example, class 2 equals an age of 1.0- to 1.5-yr-old, class 3 equals 0.9- to 3.6-yr-old, class 4 equals 2.6- to 5.4-yr-old, etc., and possums may live up to 14 yr in captivity (Kerle, 2001). Individual body weight was used as an indicator of a possum's condition over the year. Blood (3 ml) was collected from either the ventral tail vein or preferably from a jugular vein of each adult animal. Serum was separated and stored frozen at −20 C until processed. Possums were held in hanging hessian bags and released after sunset (when dark) the same day, allowing sufficient time to recover from sedation and to minimize handling-induced stress in this nocturnal mammal.

TABLE I. Individual *Toxoplasma gondii* antibody titers in sera of 142 *Trichosurus vulpecula.*

Antibody titer	No. of positive sera (%)	ID of possum
1:25	4 (2.8)	48, 96, 139, 140
1:50	1 (0.7)	184
1:100	1 (0.7)	29
1:800	1 (0.7)	141
1:3,200	1 (0.7)	72
1:6,400	1 (0.7)	101
1:12,800	1 (0.7)	101
Total positive	9 (6.3)	

Serological examinations

All serological examinations were conducted at the Animal Parasitic Diseases Laboratory, Beltsville, Maryland. Antibodies against *T. gondii* and *N. caninum* were detected using agglutination tests, which included the parasite specific antigens. For *T. gondii* the modified agglutination test (MAT) as described by Dubey and Desmonts (1987) was used and sera were diluted 2-fold starting at the 1:25 dilution. For *N. caninum*, the test was performed as described by Romand et al. (1998), and sera were screened at 1:25 serum dilution. These agglutination tests measure only IgG antibodies; therefore acute infections may not be detected. Although there is no information on the sensitivity and specificity of MAT for diagnosis of toxoplasmosis in possums, based on a validation study of the MAT in pigs naturally and experimentally infected with *T. gondii* (Dubey, 1997), a MAT titer of ≥1:25 was considered an indicator of *T. gondii* exposure and termed ''seropositive.''

Statistical analysis

The statistical analysis was performed using the Vassar Stats web site for statistical computation (http://faculty.vassar.edu/lowry/VassarStats.html). Seroprevalence was analyzed considering the variables of sex, age, and location (sampling sites). The effect of sex on infection was analyzed by binomial probabilities. For comparison of age groups and different locations the chi-square test was used. As possums were trapped over the years, the estimated age group of possums used for statistical analysis was for seropositive animals when they first tested positive and for the seronegative ones when they were last trapped. Differences were considered statistically significant when $P \leq 0.05$. However, sample sizes for infected possums were low; therefore, statistical results should be interpreted with caution.

RESULTS

Antibodies to *T. gondii* were found in 9 (6.3%) of 142 individual possums and in 10 (5.1%) of 195 serum samples. Seropositive animals were observed in nearly all suburbs: 0 (0%) of the 4 from Beecroft, 2 (14.3%) of the 14 from Chatswood West, 4 (18.2%) of the 22 in North Epping, 1 (1.6%) of the 61 in Pymble, and 2 (5.9%) of the 34 on Scotland Island, but not in Myall Lake National Park (0 [0%] of the 7). The variation in seroprevalence of *T. gondii* within the different suburbs, from 0% (Beecroft) up to 18.2% (North Epping), was not significant ($\chi^2 = 7.83$, $P = 0.1$, df = 4). Seropositivity ranged from a titer of 1:25 (possum nos. 48, 96, 139, and 140), 1:50 (no. 184), 1:100 (no. 29), 1:800 (no. 141), 1:3,200 (no. 72), and 1:6,400 (no. 101) up to a titer of 1:12,800 (no. 101) (see Table I).

Table II presents the trapping and serum sampling record of each positive tested animal. There was no apparent sex-biased

TABLE II. *Toxoplasma gondii* antibody titers of 9 affected possums including their capture records, sex, age, weight, and location.

Possum ID	MAT titer	Date of (re)capture	Sex	Age group	Weight (kg)	Location (suburb, house)
29	No sample	10 Feb 03	Female	2	1.65	Chatswood 6
	Negative	08 Oct 03		3	2.4	As above
	1:100	16 Sept 04		3	2.2	As above
	—	Not recaptured (Dec 04; Apr 05)		—	—	As above
48	Negative	26 Feb 03	Female	3	2.75	Pymble 22
	1:25	31 Oct 03		3	3.1	As above
	Negative	31 Aug 04		4	2.95	As above
72	1:3,200	25 Mar 03	Female	Not aged	2.55	Epping 5
	—	Not recaptured (Oct 03; Sept 04)		—	—	As above
96	No sample	23 Aug 03	Female	130 days	0.25	Scotland Island 35
	1:25	04 Nov 04		2–3	1.95	Scotland Island 32
101	1:12,800	27 Aug 03	Male	3	2.3	Scotland Island 34
	1:6,400	04 Nov 04		3	2.5	As above
139	1:25	15 Oct 03	Male	3	2.6	Epping 3
	—	Not recaptured (Oct 04; Apr 05)		—	—	As above
140	1:25	15 Oct 03	Male	3	2.8	Epping 3
140	—	Not recaptured (Oct 04; Apr 05)		—	—	As above
141	Negative	21 Oct 03	Male	3	2.5	Epping 2
	1:800	07 Oct 04		3	2.6	As above
184	1:50	15 Sept 04	Male	4	2.75	Chatswood 6
	—	Not recaptured (Dec 04; Apr 05)		—	—	As above

seropositivity; both females (4) and males (5) had been exposed (binomial $p = 1.0$). Seropositive possums were found mainly in the age group 3 (7 of 9 = 77.8%), which indicates an age of 0.9- to 3.6-yr-old. The majority of seronegative possums were also found in the age group 3 (43 of 133 = 32.3%) or age group 4 (32 of 133 = 24.1%). Few possums were older (16 of 142 = 11.3%), 13.4% (19 of 142) were younger (age group 2), and 16.9% (24 of 142) of adult possums from initial trapping were not aged. The differences between age groups were not significant ($\chi^2 = 8.11$, $P = 0.09$, df = 4). Body weight of seropositive converted possums usually did not decrease compared to their previous weight when seronegative (apart from no. 29), suggesting there was no loss in body condition, and they remained within the normal adult possum weight range. Five possums were not recaptured after exposure to *T. gondii* was detected (nos. 29, 72, 139, 140, 184), 1 tested negative on the third time caught (no. 48), although seropositivity of 1:25 was found on the second time caught. The other possums (nos. 96, 101, 141) were found to have *T. gondii* antibodies at the end of the sampling period, so their fate remains unknown. However, animal no. 101 was found seropositive on 27 August 2003 (1:12,800) and 4 November 2004 (1:6,400), indicating that it survived exposure to *T. gondii* for more then 14 mo. By comparison, 57–35% of the seronegative possums were retrapped on subsequent trapping events (declining recapture success with increasing time). Seropositive animals sometimes were caught on the same property, observed for nos. 29 and 184 (Chatswood 6) and nos. 139 and 140 (North Epping 3), perhaps indicating a common source of infection (see Table II). Nine householders (25.7%) owned cat(s), of which 5 had their cat(s) free-ranging in the backyard during the daytime, including house 2 North Epping and house 35 on Scotland Island.

Antibodies to *N. caninum* were not found in any of the 195 samples tested. Seven householders (20%) owned dog(s); some of these allowed their dogs to defecate frequently in the backyard.

DISCUSSION

The results of this study show that urban *T. vulpecula* are likely to be exposed to *T. gondii* in their environment, but no evidence for exposure to *N. caninum* was found. Similar or higher levels of exposure to *T. gondii* were found in studies on other Australian marsupials. A study on wild bandicoots revealed that 6.7% of trapped animals (150) had antibodies to *T. gondii* (Obendorf et al., 1996), whereas infection was found in more then 21% of 150 quokkas living near the settlement on Rottnest Island (Gibb et al., 1966). The high titers recorded from some of our samples indicated current or recent infection with *T. gondii* (see Table I). Exposure to *T. gondii* was linked to particular backyards for several possums (see Table II). Animals were found seropositive in the same backyards at both Chatswood and North Epping, yet not all possums trapped in these backyards tested seropositive. It is difficult to locate the exact source of infection as presence of free-range domestic cats and seropositive possums are not necessarily linked. Householders may have free-range cats, yet possums on these properties were seronegative, and vice versa. Possums may become infected on a neighboring property contaminated with *T. gondii* oocysts, or stray and feral cats may have shed oocysts in the affected backyard. Therefore, the exact location of infection cannot be determined as possums and cats may move through several properties having extended home ranges. The home range area for urban possums from Launceston, Tasmania, was found to be sex-dependent with females usually having an average home range of 2 ha, although males may move within a range of up to 10.9 ha (Statham and Statham, 1997). Diurnal home range areas of suburban domestic cats in Canberra ranged from 0.02 to 17.19 ha with a mean of 2.73 ha (Barratt, 1997). In general it can be presumed that opportunities for exposure to *T. gondii* are available and depend on the presence of oocysts in a particular area reflected by the varying extent of seropositive possums in different suburbs.

There was no evidence that seropositivity may be sex-linked either, although males perhaps have a greater chance of encountering oocysts because of their larger home ranges compared to females. The majority of seropositive possums were found in age group 3 (0.9- to 3.6-yr-old); however, the majority of adult possums trapped also fell into age group 3–4, and few older animals were caught. It is a common pattern for many host species and disease agents that, as an animal ages, its cumulative likelihood for exposure increases (Zarnke et al., 2000).

Seropositive possums were usually not recaptured; only 1 animal (no. 101) had antibodies to *T. gondii* on 2 bleedings 14 mo apart. Recapture success for seronegative possums declined over the sampling period from 57–35% and is influenced by further factors such as exposure to other diseases, illegal relocation of possums, road kill, and/or trap shyness (Eymann et al., 2006). One possum (no. 48) seroconverted from a positive titer of 1:25 to a negative titer; whether this change was related to technique, passively or actively acquired antibodies, or a result of the passage of time was not known. These findings are similar to those reported in bandicoots (Obendorf et al., 1996) and suggest that most animals exposed to *T. gondii* do not survive for long periods postinfection. Occasionally they may remain asymptotic. Some animals may not even survive initial infection and die of acute toxoplasmosis before IgG antibodies can be detected (Johnson et al., 1989; Canfield et al., 1990; Lynch et al., 1993; Skerratt et al., 1997).

Antibodies to *N. caninum* were not found in *T. vulpecula*. The exposure of *T. vulpecula* may depend on the presence of *N. caninum* oocysts, which may be less widespread in the environment. It is known that the dog sheds relatively few oocysts when infected (McAllister et al., 1998), but little is known at present regarding the frequency of shedding of *N. caninum* oocysts by canids, the resistance of the oocysts, and whether dogs shed oocysts more than once (Dubey, 2003). Reichel (2000) reported that 5–15% of Australian dogs had antibodies to *N. caninum*.

The difference in seroprevalence between *T. gondii* and *N. caninum* found in this study may be due to the fact that *T. gondii* is a very successful parasite, and its definitive host, the cat, can shed very large numbers of oocysts in its feces, producing substantial environmental contamination. Oocysts in the soil can be spread mechanically by flies, cockroaches, and earthworms, and a single live oocyst is enough to infect a pig orally (Dubey et al., 1996). Survival of *T. gondii* oocysts in the environment (and thus the potential reservoir of infection) may be an influencing factor on the differing seroprevalence: *T. gon-*

dii oocysts can survive for 1 yr or more (Yilmaz and Hopkins, 1972), although at present it is not known how long *N. caninum* oocysts persist in the environment. A seroprevalence study on a population of domestic cats in Melbourne showed that more then 38% of 103 tested cats had *T. gondii* positive IgG titers (Sumner and Ackland, 1999), and in a Sydney study 50% of 80 domestic cats were seropositive (Watson et al., 1982). Cats are infectious only for a short period before acquiring immunity, but millions of oocysts may be released in the feces in a single day. Although only a few cats may be shedding *T. gondii* oocysts at any given time, the enormous numbers produced and their resistance to destruction ensure widespread contamination (Dubey, 2004). Little is known of treatment and prophylaxis, and at present there is no vaccine to control toxoplasmosis in humans, cats, or wild animals (Lynch et al., 1993; Reddacliff et al., 1993; Dubey and Odening, 2001; Bhopale, 2003). Therefore control of feral cat numbers and keeping domestic cats indoors would be the only rational approach to prevent urban contamination with *T. gondii* oocysts—an approach that would also protect native urban wildlife from preying cats (Grayson and Calver, 2004).

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